

P1 1183233

REC'D 21 JUN 2004

WIPO

PCT

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

**UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office**

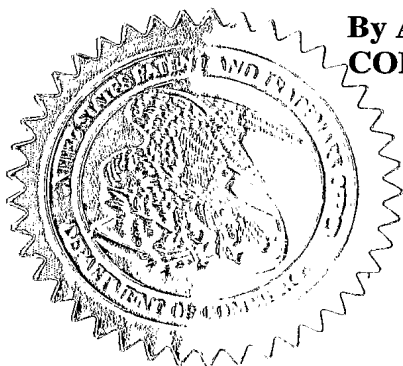
June 17, 2004

**THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM
THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK
OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT
APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A
FILING DATE.**

APPLICATION NUMBER: 60/517,961

FILING DATE: November 06, 2003

RELATED PCT APPLICATION NUMBER: PCT/US04/07733



**By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS**

N. Woodson
N. WOODSON
Certifying Officer

**PRIORITY
DOCUMENT**
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

17638 U.S. PTO

PTO/SB/16 (08-03)

Approved for use through 07/31/2006. OMB 0851-0032

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EU 730811236US

INVENTOR(S)					
Given Name (first and middle (if any))		Family Name or Surname		Residence (City and either State or Foreign Country)	
Ramesh Xiaozhong		Kekuda Qian		Stamford, CT Branford, CT	
Additional inventors are being named on the <u>1</u> separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
Novel Humanin-like Proteins and their Methods of Use					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input checked="" type="checkbox"/> Customer Number:		000037915			
OR					
<input type="checkbox"/> Firm or Individual Name					
Address					
Address					
City		State		Zip	
Country		Telephone		Fax	
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages <u>18</u>		<input type="checkbox"/> CD(s), Number _____			
<input checked="" type="checkbox"/> Drawing(s) Number of Sheets <u>6</u>		<input type="checkbox"/> Other (specify) _____			
<input type="checkbox"/> Application Date Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.				FILING FEE Amount (\$)	
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees.				80.00	
<input checked="" type="checkbox"/> The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: <u>502648</u>					
<input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.					
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input checked="" type="checkbox"/> No.					
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: _____					

[Page 1 of 2]

Respectfully submitted,

Date November 6, 2003

SIGNATURE Kausalya SanthanamREGISTRATION NO. 53,552TYPED or PRINTED NAME Kausalya Santhanam

(if appropriate)

Docket Number: Cura 946ATELEPHONE (203)974-6307

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

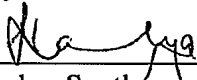
UNITED STATES PATENT AND TRADEMARK OFFICE

EXPRESS MAIL STATEMENT

Applicants' Agent Kausalya Santhanam, Ph.D. (Registration No. 53,552)
Certifies that the attached documents were deposited by her with the United States Post
Office on this 6th Day of November 2003 as a postage prepaid Express Mail filing.
(Express Mail label No. EU 730811236 US for the 18-page Provisional Patent
Application of Ramesh Kekuda and Xiaozhong Qian entitled Novel Humanin-like
Proteins and their Methods of Use

Accordingly, Applicants claim the filing date of November 6, 2003.

Certified this 6th Day of November 2003 by



Kausalya Santhanam, Ph.D. (Reg. No. 53,552)

In The United States Patent And Trademark Office

In re Application of:

Ramesh Kekuda and Xiaozhong Qian

Entitled:

Novel Humanin-like Proteins and their Methods of Use

Filed:

concurrently herewith

Mail Stop Provisional
Commissioner of Patents and Trademarks
PO Box 1450
Alexandria, VA. 22313-1450

Express Mail mailing label No. EU 730811236 US

Date of Deposit: Thursday, November 6, 2003

I hereby certify that the following papers or fees are being deposited with the United States Postal Service "Express Mail Post Office to Address" service under 37 CFR 1.10 on the date indicated above, and that these documents were addressed to:

Mail Stop Provisional
Hon. Commissioner of Patents and Trademarks
PO Box 1450
Alexandria, VA. 22313-1450

The documents deposited herein are:

- Authorization to debit Deposit Account Nos. 502648
- Letter of Transmittal
- New Provisional Patent Application comprising 18 pages
- Postal card filing receipt

PROVISIONAL PATENT APPLICATION

In the name of the inventors

Ramesh Kekuda and Xiaozhong Qian

**Novel Humanin-like Proteins
and their Methods of Use**

This application is related to Cura 941, 60/453578 filed March 11, 2003.

Novel Humanin-like Proteins and Nucleic Acids Encoding Same

The present invention discloses a novel protein and nucleic acids bearing sequence similarity to Humanin, fragments thereof, and antibodies that bind immunospecifically to a protein of the invention. The composition detailed in the present invention can be used in neurodegenerative diseases.

Background

Hashimoto et al. (2001) noted that important clues in the development of therapy for Alzheimer disease (FAD; 104300) come from the study of molecules that suppress FAD gene-induced death in neuronal cells in culture. Using the death-trap screening method devised by Vito et al. (1996), they identified a cDNA, which they called humanin (HN), encoding a deduced 24-amino acid secretory polypeptide that suppresses neuronal cell death induced by 3 FAD genes: amyloid precursor protein (APP; 104760), presenilin-1 (PS1; 104311), and presenilin-2 (PS2; 600759). The peptide also abolished death caused by A-beta amyloid, but had no effect on death by Q79 or superoxide dismutase-1 mutants. Transfected HN cDNA was transcribed to the corresponding polypeptide and then was secreted into the cultured medium. The rescue action clearly depended on the primary structure of HN. Northern blot analysis detected expression of major 1.6- and minor 3.0- and 1.0-kb transcripts at high levels in heart, skeletal muscles, kidney, and liver, at lower but significant levels in brain and the gastrointestinal tract, and at barely detectable levels in the immune system.

The cDNA sequence of HN (GenBank AY029066), is 99% identical to the sequence of 16S mitochondrial ribosomal RNA (561010), which is mitochondrially encoded, but is also 99% identical to some nuclear-encoded cDNAs. It was therefore not clear whether the HN cDNA is mitochondrial ribosomal RNA or a nuclear-transcribed mRNA. "

Four additional loci were found in human genomic sequence which encode humanin like polypeptides on chromosomes 3, 11, 17 and 5 (See the table below).

QUERY	SCORE	START	END	QSIZE	IDENTITY	CHRO	Clone	Fragment	ID
1. Humanin (AAK50430)	22	0	24	24	95.9%	3	AC117444	6	
2. Humanin (AAK50430)	22	0	24	24	95.9%	11	AC021914	7	
3. Humanin (AAK50430)	21	0	23	24	95.7%	17	AC131055	9	
4. Humanin (AAK50430)	20	0	22	24	95.5%	5	AC008434	5	

Loci on chromosome 3 and 11 encode identical polypeptides (CG202524-02) which has a S12L replacement at amino acid position 12 as compared to known humanin (AAK50430). Locus on chromosome 17 encodes another humanin related polypeptide (CG202524-03) which has 2 amino acid replacements - S12L and T24A. Locus on chromosome 5 encodes yet another humanin like polypeptide (CG202524-04) with 3 amino acid replacements - L12S, R23L and A24L. Also this has 4 additional amino acids -SSVF- at positions 25 to 28.

An interesting observation made when the 3 novel CuraGen polypeptides were compared to the known Humanin (AAK50430) is that Leu at position 12 was replaced by Ser. Hashimoto et al (2001) (reference 4) have done a systematic site-directed mutagenesis analysis of Humanin and have identified that replacing Leu-12 with Ala abolished the protective function of Humanin. However, the current invention is unique in that CG202524-02, -04 and -03 have shown protection of neurons under various conditions. This supported by the data presented in the instant application. The observation that there are 4 genomic loci which encode a Ser in place of Leu at position 12 might indicate a functional significance to this amino acid. For example, Hashimoto et al (2001) (reference 4) have shown that Ser14 substitution to Gly potentiated the neuroprotective activity of Humanin thousand fold, whereas the substitution to Ala nullified the protective activity. It is conceivable that substitution of Leu12 with Ser (found in 4 different genetic loci and found in the 3 novel Humanin variants described here) potentiates the neuroprotective activity of humanin. It is also possible that the replacement of Ala 24 with Thr in -03 variant and Arg23, Ala24 with Leu-Leu-Ser-Ser-Val-Phe in the -04 variant might have beneficial potentiating neuroprotective activities.

An additional locus was found in human genomic sequence which encode humanin like polypeptides on chromosome 6 (See the table below).

ACTIONS	QUERY	SCORE	START	END	QSIZE	IDENTITY	CHRO	STRAND	START	END
browser details	YourSeq	13	1	23	24	78.3%	6	++	62235629	
62235697										

Locus on chromosome 6 encodes another humanin related polypeptide (CG202524-08) which has 5 amino acid replacements - S12L and T24A, S14T, E15A and I16T as compared to the known humanin GenBank AY029066.

It should be noted that CG202524-08 also retains the Ser in the 12th position as compared to Leucine in the known human humanin.

REFERENCES

1. Hashimoto, Y.; Niikura, T.; Tajima, H.; Yasukawa, T.; Sudo, H.; Ito, Y.; Kita, Y.; Kawasumi, M.; Kouyama, K.; Doyu, M.; Sobue, G.; Koide, T.; Tsuji, S.; Lang, J.; Kurokawa, K.; Nishimoto, I. :
A rescue factor abolishing neuronal cell death by a wide spectrum of familial Alzheimer's disease genes and A-beta. Proc. Nat. Acad. Sci. 98: 6336-6341, 2001.
PubMed ID : 11371646
2. Vito, P.; Lacana, E.; D'Adamio, L. :
Interfering with apoptosis: Ca(2+)-binding protein ALG-2 and Alzheimer's disease gene ALG-3. Science 271: 521-524, 1996.
PubMed ID : 8560270
3. Hashimoto Y, Niikura T, Ito Y, Sudo H, Hata M, Arakawa E, Abe Y, Kita Y, Nishimoto I. :

Detailed characterization of neuroprotection by a rescue factor humanin against various Alzheimer's disease-relevant insults.
J Neurosci. 2001 Dec 1;21(23):9235-45

Comparison of the Humanin variants

```
CG202524-05-PROT 1 MAPRGFSCLLLTSE IDLPVKRRA---- 24
CG202524-01-PROT 1 MAPRGFSCLLLTSE IDLPVKRRA---- 24
CG202524-02-PROT 1 MAPRGFSCLLLTSE IDLPVKRRA---- 24
CG202524-06-PROT 1 MAPRGFSCLLLTSE IDLPVKRRA---- 24
CG202524-07-PROT 1 MAPRGFSCLLLTSE IDLPVKRLSSVF 28
CG202524-04-PROT 1 MAPRGFSCLLLTSE IDLPVKRLSSVF 28
CG202524-03-PROT 1 MAPRGFSCLLLTSE IDLPVKRRT---- 24
CG202524-08-PROT 1 MAPRGFSCLLLTSE IDLPVKRRT---- 24
```

CG202524-01 and -05 are the known humanin (GenBank: AY029066)
CG202524-02 and -06 are novel variants having the same protein sequences.
CG202524-03 is a novel variant.
CG202524-04 and -07 are novel variants having the same protein sequences.
CG202524-08 is a novel variant

Brief Description of the Drawings

Figure 1: Serum withdrawal induces PC2 cell death measured by LDH assay.

Figure 2a: HN-01 (CG202524-01) rescues PC12 cells from serum withdrawal-induced cell death.

Figure 2b: HN-06 (CG202524-02) rescues PC12 cells from serum withdrawal-induced cell death.

Figure 2c: HN-03 (CG202524-03) rescues PC12 cells from serum withdrawal-induced cell death.

Figure 2d: HN-07 (CG202524-04) rescues PC12 cells from serum withdrawal-induced cell death.

Figure 3: Effect of novel humanin variants on PC12 survival after serum withdrawal. Relative fluorescence units were normalized to the maximal fluorescence units observed under no HN treatment.

Figure 4: Effect of novel humanin variants on Dopamine-induced cell death. Relative fluorescence indicates LDH level in supernatant released from dead cells.

Figure 5: Effect of HNs on CREB-3-mediated cell death.

Table 1. Novel Humanin variants

NOVX Assignment	Internal Identification	SEQ ID NO (nucleic acid)	SEQ ID NO (amino acid)	Homology
NOV1a	CG202524-07	1	2	Humanin - Homo sapiens
NOV1b	CG202524-01	3	4	Humanin - Homo sapiens
NOV1c	CG202524-02	5	6	Humanin - Homo sapiens
NOV1d	CG202524-03	7	8	Humanin - Homo sapiens
NOV1e	CG202524-04	9	10	Humanin - Homo sapiens
NOV1f	CG202524-05	11	12	Humanin - Homo sapiens
NOV1g	CG202524-06	13	14	Humanin - Homo sapiens
NOV1h	CG202524-08	15	16	Humanin - Homo sapiens

Example 1

The NOV1 clone was analyzed, and the nucleotide and encoded polypeptide sequences are shown in Table 1A.

Table 1A. NOV1 Sequence Analysis			
NOV1a, CG202524-07 DNA Sequence	SEQ ID NO: 1		90 bp
	ORF Start: ATG at 4		ORF Stop: TAG at 88
ACCATGGCTCCACGAGGGTTCAGCTGTCTCTTACTTTCAACCAGTGAAATTGACCTGCCCGTGAAGAG ACTTTTAAAGTTCAGTTTTTTTAG			
NOV1a, CG202524-07 Protein Sequence	SEQ ID NO: 2	28 aa	MW at 3080.6kD
	MAPRGFSCLLLSTSEIDL PVKRL LSSVF		
NOV1b, CG202524-01 DNA Sequence	SEQ ID NO: 3		1567 bp
	ORF Start: ATG at 951		ORF Stop: TAA at 1023
CCCAAACCCACTCCACCTTACTACCAGACAACCTTAGCCAAACCATTTACCCAAATAAAGTATAGGCG ATAGAAATTGAAACCTGGCGCAATAGATATAGTACCGCAAGGGAAAAGATGAAAAATTATAACCAAGCA TAATATAGCAAGGACTAACCCCTATACCTTCTGCATAATGAATTAAGTAAATAAAGTATAGGCG AGCCAAAGCTAAGACCCCGAAACCAGACGAGCTACCTAAGAACAGCTAAAAGAGGCACACCCGCTCTAT GTAGCAAAATAGTGGGAAGATTATAGGTAGAGGCGACAAACCTACCGAGCCTGGTGATAGCTGGTTG TCCAAGATAGAAATCTTAGTTCAACTTTAAATTTGCCACAGAACCCCTCTAAATCCCTTGTAATTTTA ACTGTTAGTCCAAAGAGGAACAGCTCTTTGGACACTAGGAAAAAACCTTG TAGAGAGAGTAAAAAATT TAACACCCATAGTAGGCCATAAAGCAGCCACCAATTAAGAAAGCGTTCAAGCTCAACACCCACTACCT AAAAAATCCCAACATATAACTGAACTCCTCACACCCCAATTGGACCAATCTATCACCCTATAGAAGAA CTAATGTTAGTATAAGTAACATGAAAACATTCCTCCGCATAAGCCTGCGTCAGATTAAACACTGA			

ACTGACAATTAACAGCCCAATATCTACAATTAACCAACAAGTCATTATTACCCTCACTGTCAACCCAA
CACAGGCATGCTCATAAGGAAAGGTTAAAAAAGTAAAGGAACTCGGCAAATCTTACCCCGCCTGTT
TACCAAAAACATCACCTCTAGCATCACCAGTATTAGAGGCACCGCCTGCCAGTGACACATGTTTAAAC
GGCCGCGGTACCCTAACCGTGCAAAGGTAGCATAATCACTTGTTCCTTAATTAGGGACCTGTATGAAT
GGCTCCACGAGGGTTTCAGCTGTCTCTTACTTTTAAACAGTGAAATTGACCTGCCCGTGAAGAGGCGGG
CATAACACAGCAAGACGAGAAGACCCTATGGAGCTTTAATTTATTAATGCAAACAGTACCTAACAAAC
CCACAGGTCCTAAACTACCAAACCTGCATTAAAAATTTTCGGTTGGGGCGACCTCGGAGCAGAACCCAA
CCTCCGAGCAGTACATGCTAAGACTTCACCAGTCAAAGCGAACTACTATACTCAATTGATCCAATAAC
TTGACCAACGGAACAAGTTACCCTAGGGATAACAGCGCAATCCTATTCTAGAGTCCATATCAACAATA
GGGTTTACAGCTCGATGTTGGATCAGGACATCCCAATGGTGCAGCCGCTATTAAAGGTTTCGTTTGT
AACAGATTAAAGTCTACGTGATCTGAGTTACAGACCGGAGTAATCCAGGTCGGTTTCTATCTACTTCA
AATTCTCTCCCTGTACGAAAGGACAAGAGAAATAAGGCCTACTTCACAAAGCGCCTTCCCCCGTAAATG
ATATCATCTCAACTTAGTATTATACCCACACCCACCCAAGAACAGGGTTTGTTAAGAAAAAAAAAAAA
AAA

NOV1b, CG202524-01 SEQ ID NO: 4 24 aa MW at 2687.2kD

Protein Sequence

MAPRGFSCLLLLTSEIDL PVKRRRA

NOV1c, CG202524-02

SEQ ID NO: 5

75 bp

DNA Sequence

ORF Start: ATG at 1

ORF Stop: TAA at 73

ATGGCTCCACGAGGGTTCAGCTGTCTCTTACTTTCAACCAGTGAAATTGACCTACCCGTGAAGAGGCG
GGCATAA

NOV1c, CG202524-02 SEQ ID NO: 6 24 aa MW at 2661.1kD

Protein Sequence

MAPRGFSCLLLLTSEIDL PVKRRRA

NOV1d, CG202524-03

SEQ ID NO: 7

75 bp

DNA Sequence

ORF Start: ATG at 1

ORF Stop: TAA at 73

ATGGCTCCACGAGGGTTCAGCTGTCTCTTACTTTCAACCAGTGAAATTGACCTGCCCGTGAAGAGGCG
GACATAA

NOV1d, CG202524-03 SEQ ID NO: 8 24 aa MW at 2691.1kD

Protein Sequence

MAPRGFSCLLLLTSEIDL PVKRRT

NOV1e, CG202524-04

SEQ ID NO: 9

87 bp

DNA Sequence

ORF Start: ATG at 1

ORF Stop: TAA at 85

ATGGCTCCACGAGGGTTCAGCTGTCTCTTACTTTCAACCAGTGAAATTGACCTGCCCGTGAAGAGACT
TTTAAGTTTCAGTTTTTTTAA

NOV1e, CG202524-04 SEQ ID NO: 10 28 aa MW at 3080.6kD

Protein Sequence

MAPRGFSCLLLLTSEIDL PVKRLLSVF

NOV1f, CG202524-05

SEQ ID NO: 11

78 bp

DNA Sequence

ORF Start: ATG at 4

ORF Stop: TAG at 76

ACCATGGCTCCACGAGGGTTCAGCTGTCTCTTACTTTTAAACCAGTGAAATTGACCTGCCCGTGAAGAG
GCGGGCATAG

NOV1f, CG202524-05 SEQ ID NO: 12 24 aa MW at 2687.2kD

Protein Sequence

MAPRGFSCLLLLTSEIDL PVKRRRA

NOV1g, CG202524-06

SEQ ID NO: 13

79 bp

DNA Sequence

ORF Start: ATG at 4

ORF Stop: TAG at 76

ACCATGGCTCCACGAGGGTTCAGCTGTCTCTTACTTTCAACCAGTGAAATTGACCTACCCGTGAAGAGCGGGGCATAGC			
NOV1g, CG202524-06	SEQ ID NO: 14	24 aa	MW at 2661.1kD
Protein Sequence			
MAPRGFSCLLLTSEIDLVPKRRA			
NOV1h, CG202524-08	SEQ ID NO: 15	75 bp	
DNA Sequence		ORF Start: ATG at 1	ORF Stop: TAA at 73
ATGGGCTCGACGAGGTTTCAGCTGTCTCTTACTTTCAACCAGTGCAACTGACCTGCCCGTGAAGAGGCGGACATAA			
NOV1h, CG202524-08	SEQ ID NO: 16	24 aa	MW at 2694.2kD
Protein Sequence			
MARRGFSCLLLTSTATDLPVKRRT			

A ClustalW comparison of the above protein sequences yields the following sequence alignment shown in Table 1B.

Table 1B. Comparison of the NOV1 protein sequences.	
NOV1a	MAPRGFSCLLLTSEIDLVPKRLLSSVF
NOV1b	MAPRGFSCLLLTSEIDLVPKRRA----
NOV1c	MAPRGFSCLLLTSEIDLVPKRRA----
NOV1d	MAPRGFSCLLLTSEIDLVPKRRT----
NOV1e	MAPRGFSCLLLTSEIDLVPKRLLSSVF
NOV1f	MAPRGFSCLLLTSEIDLVPKRRA----
NOV1g	MAPRGFSCLLLTSEIDLVPKRRA----
NOV1h	MARRGFSCLLLTSTATDLPVKRRT----
NOV1a	(SEQ ID NO: 2)
NOV1b	(SEQ ID NO: 4)
NOV1c	(SEQ ID NO: 6)
NOV1d	(SEQ ID NO: 8)
NOV1e	(SEQ ID NO: 10)
NOV1f	(SEQ ID NO: 12)
NOV1g	(SEQ ID NO: 14)
NOV1h	(SEQ ID NO: 16)

Further analysis of the NOV1a protein yielded the following properties shown in Table 1C.

Table 1C. Protein Sequence Properties NOV1a	
SignalP analysis:	No Known Signal Sequence Predicted
PSORT II analysis:	
PSG: a new signal peptide prediction method N-region: length 4; pos.chg 1; neg.chg 0 H-region: length 10; peak value 8.62 PSG score: 4.22	
GvH: von Heijne's method for signal seq. recognition GvH score (threshold: -2.1): -6.20 possible cleavage site: between 18 and 19	
>>> Seems to have no N-terminal signal peptide	

ALOM: Klein et al's method for TM region allocation
Init position for calculation: 1
Tentative number of TMS(s) for the threshold 0.5: 0
number of TMS(s) .. fixed
PERIPHERAL Likelihood = 6.47 (at 8)
ALOM score: 6.47 (number of TMSs: 0)

MTOP: Prediction of membrane topology (Hartmann et al.)
Center position for calculation: 6
Charge difference: -2.0 C(0.0) - N(2.0)
N >= C: N-terminal side will be inside

MITDISC: discrimination of mitochondrial targeting seq
R content: 1 Hyd Moment(75): 9.77
Hyd Moment(95): 7.99 G content: 1
D/E content: 2 S/T content: 4
Score: -4.68

Gavel: prediction of cleavage sites for mitochondrial preseq
R-2 motif at 14 PRG|FS

NUCDISC: discrimination of nuclear localization signals
pat4: none
pat7: none
bipartite: none
content of basic residues: 10.7%
NLS Score: -0.47

KDEL: ER retention motif in the C-terminus: none

ER Membrane Retention Signals:
XXRR-like motif in the N-terminus: APRG
none

SKL: peroxisomal targeting signal in the C-terminus: none

PTS2: 2nd peroxisomal targeting signal: none

VAC: possible vacuolar targeting motif: none

RNA-binding motif: none

Actinin-type actin-binding motif:
type 1: none
type 2: none

NMYR: N-myristoylation pattern : none

Prenylation motif: none

memYQRL: transport motif from cell surface to Golgi: none

Tyrosines in the tail: none

Dileucine motif in the tail: none

checking 63 PROSITE DNA binding motifs: none

checking 71 PROSITE ribosomal protein motifs: none

checking 33 PROSITE prokaryotic DNA binding motifs: none

NNCN: Reinhardt's method for Cytoplasmic/Nuclear discrimination
Prediction: nuclear
Reliability: 55.5

COIL: Lupas's algorithm to detect coiled-coil regions
total: 0 residues

Final Results (k = 9/23):

60.9 %: nuclear
17.4 %: mitochondrial
13.0 %: extracellular, including cell wall
8.7 %: cytoplasmic

>> prediction for CG202524-07 is nuc (k=23)

A search of the NOV1a protein against the Geneseq database, a proprietary database that contains sequences published in patents and patent publication, yielded several homologous proteins shown in Table 1D.

Table 1D. Geneseq Results for NOV1a

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	NOV1a Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAO30314	Human humanin (cytosolic form) peptide - Homo sapiens, 24 aa. [WO2003046205-A2, 05-JUN-2003]	1..22 1..22	21/22 (95%) 21/22 (95%)	1e-04
AAO30161	Human humanin protein, HN1 - Homo sapiens, 24 aa. [WO2003045988-A2, 05-JUN-2003]	1..22 1..22	21/22 (95%) 21/22 (95%)	1e-04
AAU69614	Cell death protective sequence CNI-00725, protein #4 - Homo sapiens, 24 aa. [WO200176532-A2, 18-OCT-2001]	1..22 1..22	21/22 (95%) 21/22 (95%)	1e-04
ABB44628	Human protective sequence CNI-00734 peptide #5 - Homo sapiens, 24 aa. [WO200176457-A2, 18-OCT-2001]	1..22 1..22	21/22 (95%) 21/22 (95%)	1e-04
AAU73274	Human protective DNA sequence CNI-00736 open reading frame #5 - Homo sapiens, 24 aa. [WO200181361-A1, 01-NOV-2001]	1..22 1..22	21/22 (95%) 21/22 (95%)	1e-04

In a BLAST search of public sequence databases, the NOV1a protein was found to have homology to the proteins shown in the BLASTP data in Table 1E.

Table 1E. Public BLASTP Results for NOV1a

Protein Accession Number	Protein/Organism/Length	NOV1a Residues/ Match Residues	Identities/ Similarities for the Matched Portion	Expect Value
Q8IVG9	Humanin - Homo sapiens (Human), 24 aa.	1..22 1..22	21/22 (95%) 21/22 (95%)	2e-04

Description of the Invention

Method of Identifying the Nucleic Acid Encoding the Humanin-Like Protein

The sequences of Acc. No. CG202524-02, CG202524-03 and CG202524-04 was derived by laboratory cloning of cDNA fragments, by *in silico* prediction of the sequence. cDNA fragments covering either the full length of the DNA sequence, or part of the sequence, or both, were cloned. *In silico* prediction was based on sequences available in Curagen's proprietary sequence databases or in the public human sequence databases, and provided either the full length DNA sequence, or some portion thereof.

Variant sequences are also included in this application. A variant sequence can include a single nucleotide polymorphism (SNP). A SNP can, in some instances, be referred to as a "cSNP" to denote that the nucleotide sequence containing the SNP originates as a cDNA. A SNP can arise in several ways. For example, a SNP may be due to a substitution of one nucleotide for another at the polymorphic site. Such a substitution can be either a transition or a transversion. A SNP can also arise from a deletion of a nucleotide or an insertion of a nucleotide, relative to a reference allele. In this case, the polymorphic site is a site at which one allele bears a gap with respect to a particular nucleotide in another allele. SNPs occurring within genes may result in an alteration of the amino acid encoded by the gene at the position of the SNP. Intragenic SNPs may also be silent, when a codon including a SNP encodes the same amino acid as a result of the redundancy of the genetic code. SNPs occurring outside the region of a gene, or in an intron within a gene, do not result in changes in any amino acid sequence of a protein but may result in altered regulation of the expression pattern. Examples include alteration in temporal expression, physiological response regulation, cell type expression regulation, intensity of expression, and stability of transcribed message.

One or more genomic clones AC117444.6 on chromosome 3, AC021914.7 on chromosome 11, AC131055.9 on chromosome 17, and AC008434.5 Chromosome 5 were identified by TBLASTN using CuraGen Corporation's sequence file for members of Humanin and/or the Neuroprotective factor family, run against the genomic daily files made available by GenBank or obtained from Human Genome Project Sequencing centers. These sequences were analyzed for putative coding regions as well as for similarity to known DNA and protein sequences. Programs used for these analyses

include Grail, Genscan, BLAST, HMMER, FASTA, Hybrid and other relevant programs. Putative coding regions were spliced from the genomic clone and then concatenated using a known homolog for reference. The derived sequence may have been further extended using additional genomic clones showing greater than 98% identity to the open reading frame.

The regions defined by the procedures described above were then manually integrated and corrected for apparent inconsistencies that may have arisen, for example, from miscalled bases in the original fragments or from discrepancies between predicted exon junctions, and regions of sequence similarity, to derive the final sequence disclosed herein. When necessary, the process to identify and analyze genomic clones was reiterated to derive the full length sequence. The following public components were thus included in the invention: AY029066.1.

The DNA sequence was analyzed to identify any open reading frames encoding novel full length proteins as well as novel splice forms of these genes. The DNA sequence and protein sequence for a novel Humanin-like gene are reported here as CuraGen Acc. No. CG202524-02 (same as -06), CG202524-03 and CG202524-04 (same as -07) and CG202524-08.

Uses of the Compositions of the Invention

The protein similarity information, expression pattern, cellular localization, and map location for the protein and nucleic acid disclosed herein suggest that this Humanin-like protein may have important structural and/or physiological functions characteristic of the Neuroprotective factor family. Therefore, the nucleic acids and proteins of the invention are useful in potential diagnostic and therapeutic applications and as a research tool. These include serving as a specific or selective nucleic acid or protein diagnostic and/or prognostic marker, wherein the presence or amount of the nucleic acid or the protein are to be assessed. These also include potential therapeutic applications such as the following: (i) a protein therapeutic, (ii) a small molecule drug target, (iii) an antibody target (therapeutic, diagnostic, drug targeting/cytotoxic antibody), (iv) a nucleic acid useful in gene therapy (gene delivery/gene ablation), (v) an agent promoting tissue regeneration *in vitro* and *in vivo*, and (vi) a biological defense weapon.

The nucleic acids and proteins of the invention have applications in the diagnosis and/or treatment of various diseases and disorders. For example, the compositions of the present invention will have efficacy for the treatment of patients suffering from: Alzheimer's disease, Stroke, Tuberous sclerosis, hypercalcaemia, Parkinson's disease, Huntington's disease, Cerebral palsy, Epilepsy, Lesch-Nyhan syndrome, Multiple sclerosis, Ataxia-telangiectasia, Leukodystrophies, Behavioral disorders, Addiction, Anxiety, Pain, Neuroprotection, Cardiomyopathy, Atherosclerosis, Hypertension, Congenital heart defects, Aortic stenosis, Atrial septal defect (ASD), Atrioventricular (A-V) canal defect, Ductus arteriosus, Pulmonary stenosis, Subaortic stenosis, Ventricular septal defect (VSD), valve diseases, Tuberous sclerosis, Scleroderma, Obesity, Transplantation, Diabetes, Autoimmune disease, Renal artery stenosis, Interstitial nephritis,

Glomerulonephritis, Polycystic kidney disease, Systemic lupus erythematosus, Renal tubular acidosis, IgA nephropathy, Hypercalcaemia, Lesch-Nyhan syndrome, Von Hippel-Lindau (VHL) syndrome, Cirrhosis as well as other diseases, disorders and conditions.

The data included herein support the use of the nucleic acids, polypeptides and antibodies in neurodegenerative diseases as described above.

Example 1

Serum withdrawal-induced toxicity

The therapeutic potential of humanin-like polypeptides for neurodegenerative diseases was evaluated in vitro. Serum is critical for in vitro growth and survival of several populations of primary neurons and neuronal cell lines, such as PC12 cells. It has been used broadly as a primitive neuronal cell death model. PC12 cell is a rat pheochromocytoma cell line that has been widely used as a cell system for neuronal signaling and differentiation due to its ability to alter its phenotype to a sympathetic neuron-like cell in response to nerve growth factor or fibroblast growth factor. Therefore, determining whether the CG202524 variants rescued PC12 from cell death induced by serum withdrawal provided insight on the role of these proteins in neuronal protection.

A rapid, fluorescence-based LDH assays was used to measure the release of lactate dehydrogenase from cells with a damaged membrane. Briefly, PC12 cells were plated in 96 wells plate in complete serum. After 24 hours, cells were washed with serum-free media twice and were cultured in serum-free media together with a CG202524 variants at various concentrations. Cell supernatants were collected and subjected to LDH assays (CytoTox-ONE™, Promega, WI) at 24 or 48 hrs after treatment. The reactions were terminated by adding a stop buffer and the signal measured using a fluorescence reader. Higher read of LDH signal indicated more cell death occurring during the incubation time.

Results Figure 1 shows the cell death response pattern of PC-12 upon serum starvation. The data presented indicates that serum starvation results in a time dependent increase in the PC-12 cell death. However, Humanin HN-01 (CG202524-01, known form) protein rescued the PC-12 cells from serum starvation induced cell death (Figure 2a). Similar results were obtained with the novel humanin variants HN-06 (CG202524-02 also known as -06), HN-03 (CG202524-03), HN-07 (CG202524-04 also known as -07) as seen in Figures 2b, 2c and 2d respectively. Also the effect of survival of PC-12 was comparable in the presence of all the novel variants identified (Figure 3). These results collectively suggest that the novel variants described herein have the capability to protect neurons and thus can be effective as therapeutics for neurodegenerative diseases.

Example 2

Dopamine-induced toxicity

Dopamine induces cell toxicity in primary neurons and several neuronal cell lines, such as PC12 cells. It has been implicated that Dopamine toxicity is partially responsible for neuronal cell death in Alzheimer's, Parkinson's disease and several other neurodegenerative diseases. Therefore, determining whether a Humanin-like variants described herein, rescues PC12 cell death induced by Dopamine will provide important information about the role of the proteins in neuron protection.

A rapid, fluorescence-based LDH assay was used to measure the release of lactate dehydrogenase from PC-12 cells with a damaged membrane as described in Example 1. Again, all the humanin variants tested, HN-01, HN-06, HN-03, HN-07, showed comparable efficiency in protecting the PC-12 cells from dopamine-induced toxicity (Figure 4). These results further emphasize the role of the novel variants in neuronal protection and the therapeutic potential of the peptides in neurodegenerative diseases.

Example 3 **CREB-3 mediated toxicity**

The goal of this experiment was to assess the survival of PC-12 cells in the presence of Humanin-like polypeptides (CG202524-01, CG202524-02, CG202524-04). Cells were transiently cotransfected with a dominant interfering CREB-3 plasmid and plasmids encoding different isoforms of HNs. LDH level in supernatant released from dead cells was determined by LDH assay. The control without humanin polypeptide showed cell death, while in the presence of humanin variants, the cells were rescued suggesting the protective role of humanin variants in CREB-3 mediated cytotoxicity (Figure 5).

Figure 1

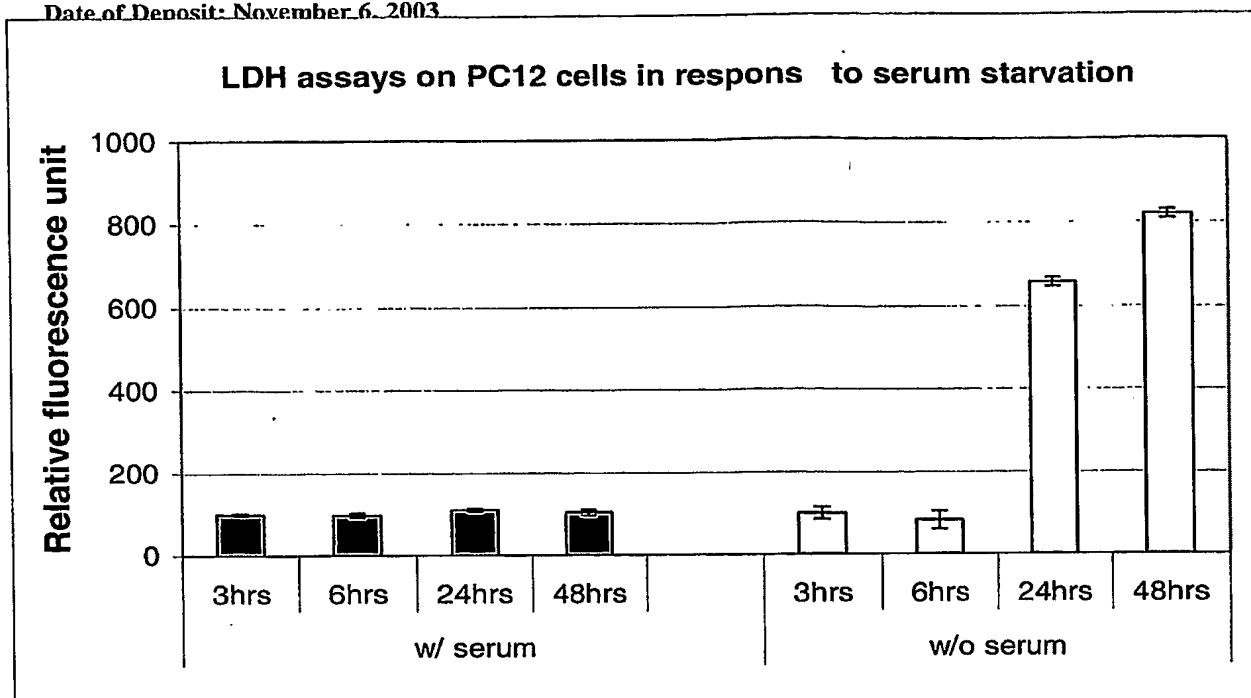


Figure 2a

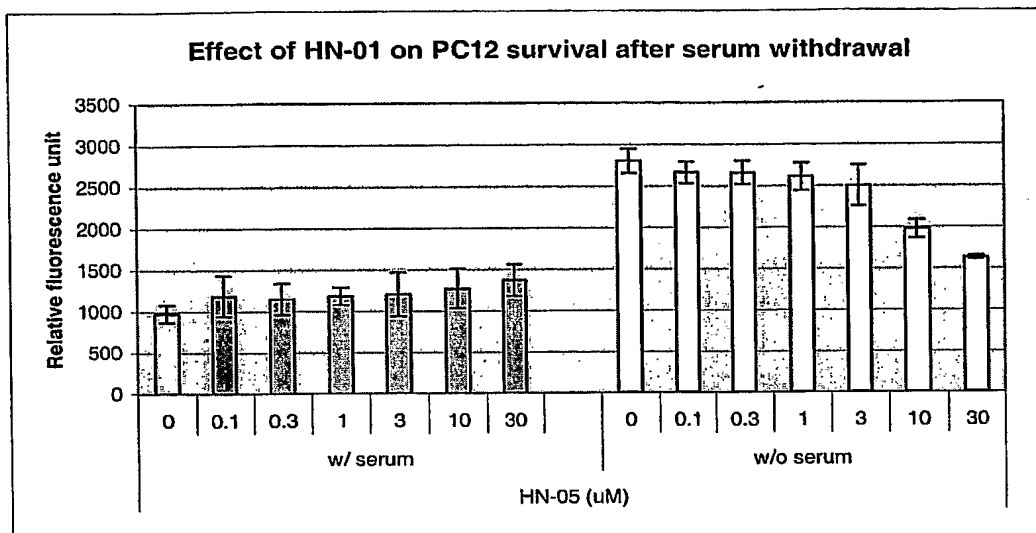


Figure 2b

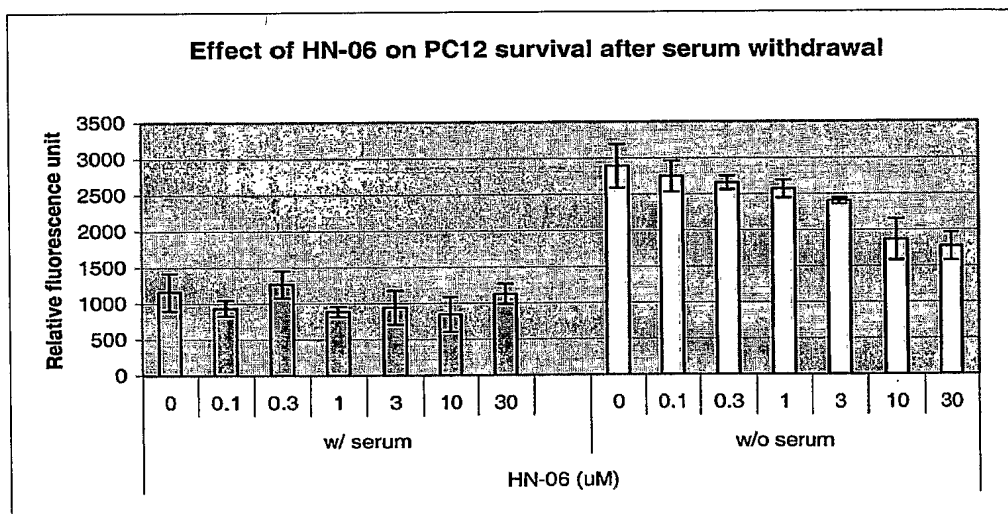


Figure 2c

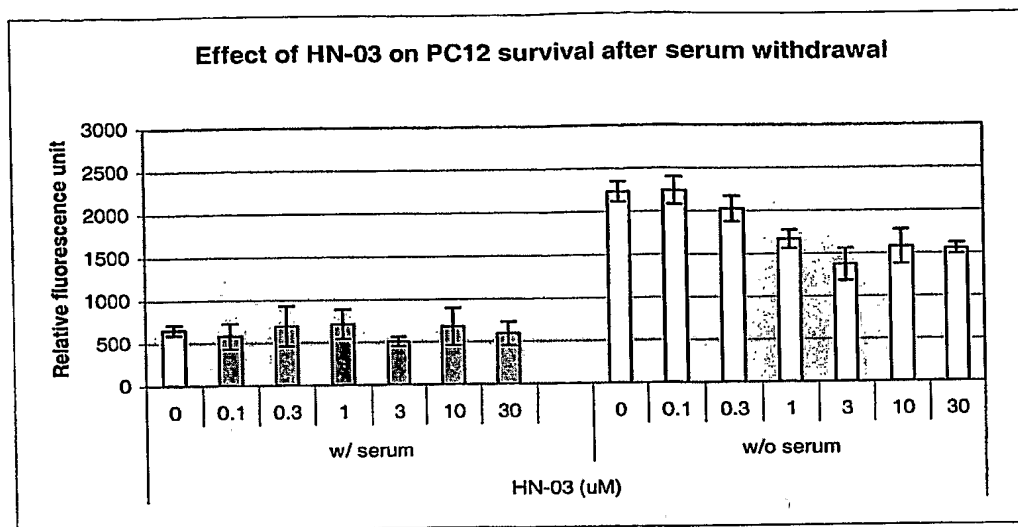


Figure 2d

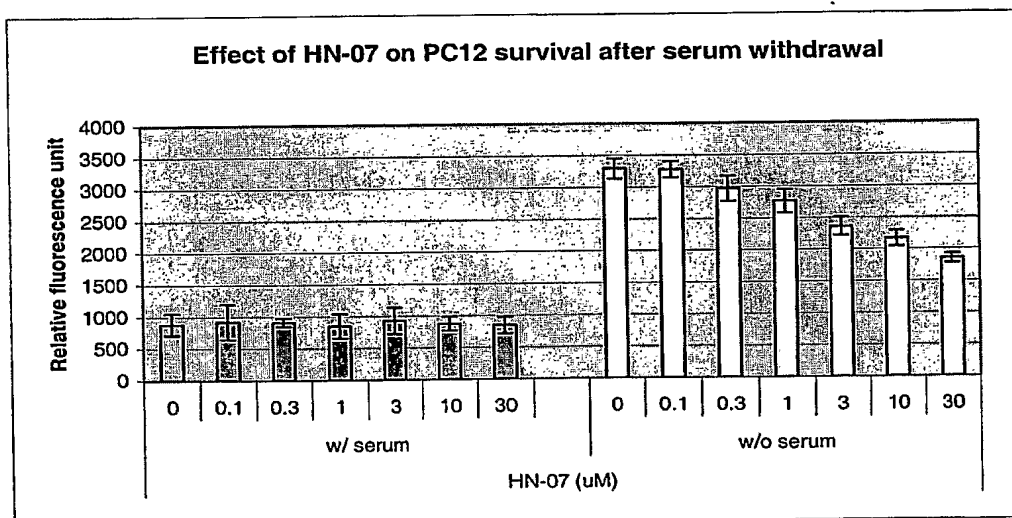


Figure 3

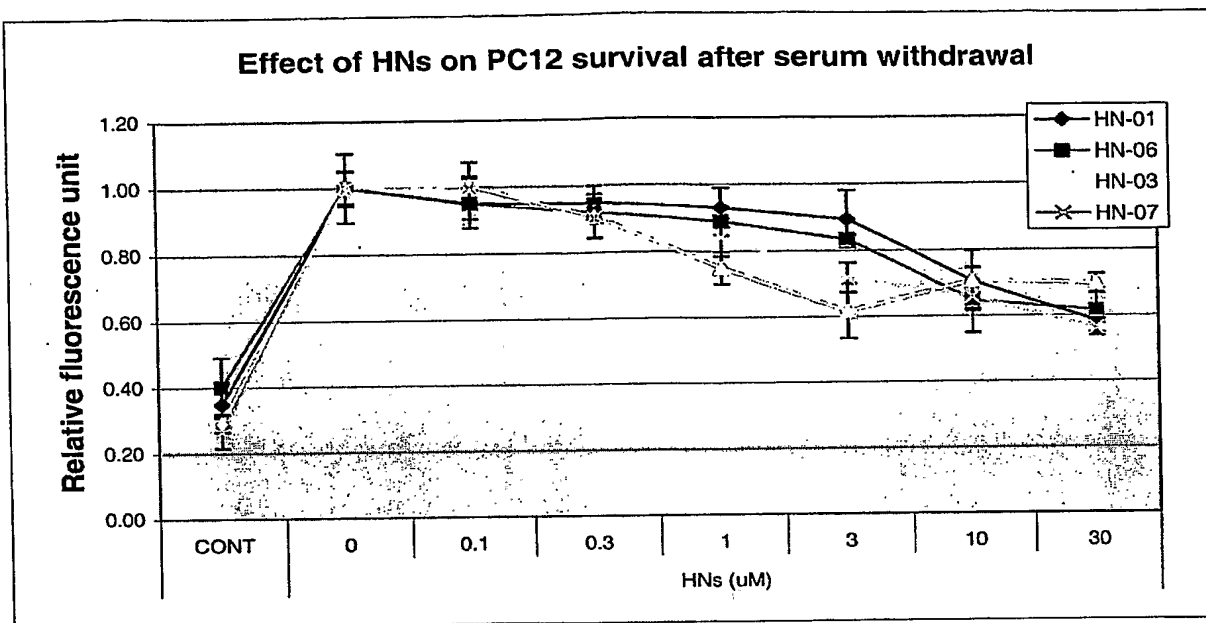


Figure 4

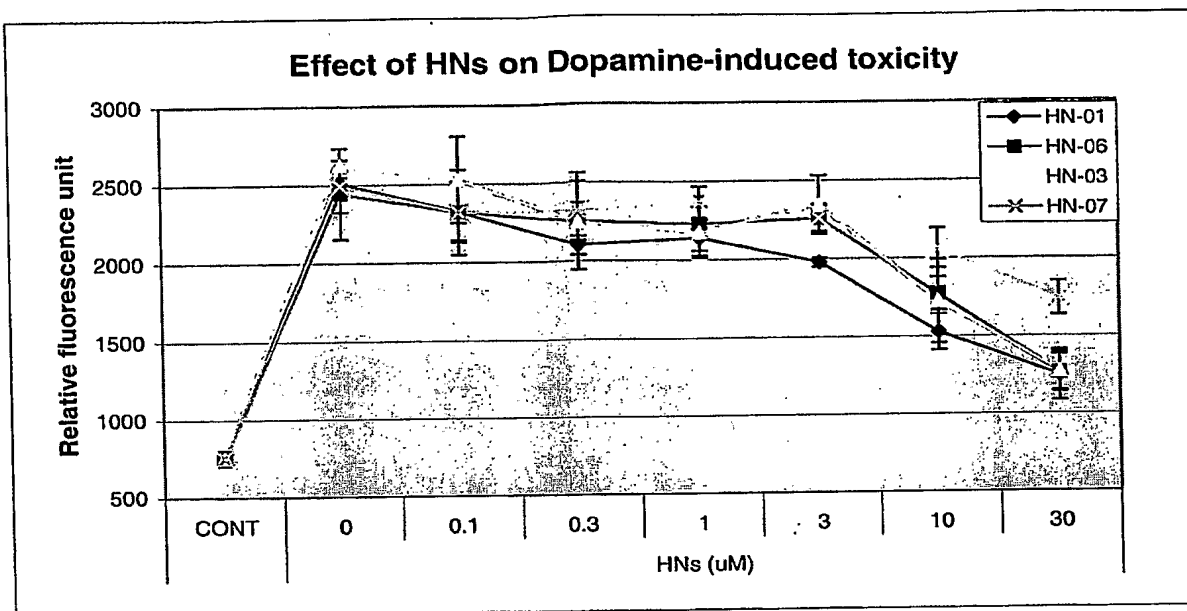


Figure 5

